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Effect of Ozone on Drug-Induced Sleeping Time in Mice Pretreated with Mixed-Function Oxidase Inducers and Inhibitors

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16. ABSTRACT

Studies were conducted to investigate the effect of ozone in prolonging pentobarbital (PEN)-induced sleeping time (S.T.). Since ozone is a common air pollutant, an ozone-induced alteration of mechanisms of drug action could have public health implications It was shown that a 5-hr exposure to 1960 ug ozone/m^3 (1 ppm) caused an increased PEN-induced S.T. in female mice (three strains), rats, and hamsters. This response was not observed in male rats or in male mice even when the latter were exposed for 5 hr/day for 3 days. Male hamsters were affected, but less so than females. Ozone concentration and time relationships were investigated in female mice. Concentrations from 196 to 9800 ug ozone/m^3 (0.1 to 5 ppm) increased PEN-induced S.T. However, as the concentration was decreased, an increasing number of daily 3-hr exposures were needed to cause an effect. Once the maximal effect was observed, further daily exposures resulted in a dissipation or a disappearance of the effect. Also, recovery occurred within 24 hr after exposure ceased. Other experiments were eprformed in which mice received a continuous exposure to a C X T (concentration x time) of 5, ranging from 196 ug/m^3 (0.1 ppm) x 50 hr to 1960 ug/m^3 (1 ppm x 5 hr. For most of the concentrations, the magnitudes of the ozone effects were roughly equivalent. These data are interpreted as a systemic effect of ozone on mechanisms of the termination of action of pentobarbital.

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Effect of Ozone on Drug-Induced Sleeping Time in Mice Pretreated with Mixed-Function Oxidase Inducers and Inhibitors[1,2]

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Effect of Ozone on Drug-Induced Sleeping Time in Mice Pretreated with Mixed-Function Oxidase Inducers and Inhibitors. GRAHAM, J. A., MENZEL, D. B., MILLER, F. J., ILLING, J. W., AND GARDNER, D. E. (1982). *Toxicol. Appl. Pharmacol.* **62**, 489–497. Prior studies have shown that ozone (O_3) increases pentobarbital (PEN)-induced sleeping time (S.T.) in female mice, rats, and hamsters. To investigate some potential mechanisms producing these effects, we measured zoxazolamine-induced paralysis time and thiopental- and hexobarbital-induced S.T., all of which were prolonged significantly in mice following a 5-hr exposure to 1960 μg O_3/m^3 (1 ppm). To probe the effect of O_3 exposure on the drug metabolism microsomal monooxygenase system, CD-1 mice were pretreated with mixed-function oxidase inducers [PEN, phenobarbital, pregnenolone-16α-carbonitrile, and β-naphthoflavone (BNF)] or inhibitors (SKF 525A and piperonyl butoxide) prior to a 5-hr exposure to 1960 μg O_3/m^3 (1 ppm). In CD-1 mice, pretreatment with PEN caused no effects on PEN-induced S.T. For all other compounds, the PEN-induced S.T. of CD-1 mice was significantly decreased, irrespective of whether the animals were exposed to air or O_3. Ozone exposure significantly increased PEN-induced S.T. equivalently in the vehicle- and inducer-pretreated animals. Similar studies were conducted in DBA and C57BL mice pretreated with BNF. DBA mice responded similarly to CD-1 mice. However, with C57BL mice, O_3 significantly increased PEN-induced S.T. in the vehicle, but not in the BNF group. When CD-1 mice were pretreated with inhibitors, a generally increased magnitude of the O_3 effect was observed with increasing doses of inhibitor. These data suggest that O_3 exposure may affect several aspects of drug metabolism and distribution.

Ozone (O_3) increases pentobarbital (PEN)-induced sleeping time (S.T.) in female mice, rats, and hamsters (Gardner *et al.* 1974; Graham *et al.*, 1981). Concentration- and time–response studies with mice have shown that this effect is observable at ambient concentrations of O_3, as low as 196 $\mu g/m^3$ (0.1 ppm) for 3 hr/day for 16 days or for 50 hr of continuous exposure. Therefore, additional studies were conducted to elucidate the mechanism of O_3 action.

To determine whether this effect of O_3 was specific to PEN, other drugs (hexobarbital, zoxazolamine, and thiopental) with different mechanisms of termination of ac-

[1] This research has been presented in part at the Annual Meeting of the Society of Toxicology, Washington, D.C., 1980, and was submitted to the Department of Physiology and Pharmacology, Duke University, in partial fulfillment of the requirements for a Ph.D. degree.

[2] This report has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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tion were studied. Hepatic aliphatic hydroxylation of PEN is the major mechanism responsible for termination of its drug action (Brodie, 1956; Cooper and Brodie, 1957; Fouts *et al.*, 1961; Ossenberg *et al.*, 1975). Thiopental undergoes an aliphatic hydroxylation by mixed-function oxidases (MFO) (Cooper and Brodie, 1957), but redistribution of the drug is a more significant factor than metabolism in termination of its hypnotic action (Goldstein and Aronow, 1960; Mark, 1963; Price *et al.*, 1960). Hexobarbital induces a S.T. of moderate duration because of rapid metabolism (aliphatic hydroxylation), rather than tissue redistribution of the drug (Bush and Weller, 1972). The major metabolic pathway for zoxazolamine is hydroxylation of the aromatic nucleus (Burns *et al.*, 1958; Tomasewski *et al.*, 1976; Robinson and Nebert, 1974; Conney *et al.*, 1960a,b).

To assess whether O_3 interfered with PEN metabolism, thereby resulting in increased PEN-induced S.T., several MFO enzyme inducers and inhibitors (SKF 525A and piperonyl butoxide) with partially known mechanisms of action were studied utilizing pretreatment prior to O_3 exposure and measurement of PEN-induced S.T. β-Naphthoflavone (BNF), pregnenolone-16α-carbonitrile (PCN), and phenobarbital (PHE) were chosen based upon the research of Haugen *et al.* (1976) who experimented with 4- to 6-week-old female C57BL/6N (Ah^b/Ah^b, responsive to aromatic hydrocarbons) and DBA/2N (Ah^d/Ah^d, nonresponsive) inbred mice, as were used in the BNF studies to be described here. These investigators found that BNF, PCN, and PHE differentially induced four isoenzyme forms of hepatic cytochromes *P*-450, but there were no major differences in any of the enzymes or electrophoretically identified hemoproteins studied between C57BL and DBA control mice. Major strain differences were only observed after BNF treatment, the greatest of which was the induction of cytochrome *P*-448-related enzymes in C57BL, but not DBA mice.

METHODS

Animals. Female CD-1 (5 to 6 weeks old), C57BL/6N (6 to 7 weeks old), and DBA/2N (6 to 7 weeks old) mice were purchased from Charles River Farms, Inc. (Wilmington, Mass.) and were maintained on a bedding of white pine shavings until use (between 6 and 11 days after receipt). They received Wayne Lab Blox and water *ad libitum* except during O_3 exposure. A 12-hr photoperiod was maintained.

Measurements of sleeping or paralysis time. S.T. studies were conducted as described by Graham *et al.* (1981). These, as well as paralysis time studies, were begun routinely at 1:00 PM and were conducted in an isolated temperature (24 ± 1°C) and humidity (46 ± 2% RH) controlled room. All injections were im in an approximate volume of 0.2 ml. Sodium pentobarbital (Nembutal, Abbott Laboratories, Chicago, Ill.) was diluted 1:10 in physiological saline, and a 50 mg/kg dose was given. Hexobarbital (Sigma Chemical Co., St. Louis, Mo.) which had been dissolved in 10% ethanol and 67.8% propylene glycol in water and diluted 1:10 with physiological saline was given at a dose of 60 mg/kg. Other experiments were conducted with mice injected with 6.25 mg/kg sodium thiopental (Pentothal, Abbott Laboratories, Stone Mountain, Ga.) which had been diluted 1:4 in physiological saline. Both induction time (I.T.) (time between injection and the animal remaining on its back, i.e., loss of righting reflex) and S.T. (the length of time elapsed between the loss and regaining of the righting reflex) were measured. I.T. and paralysis time (time between loss and regaining of the righting reflex consequent to leg paralysis) were measured in mice injected with 60 mg/kg zoxazolamine (2-amino-5-chlorobenzoxazole, Aldrich Chemical Co., Inc., Milwaukee, Wisc.) which had been dissolved in 10% ethanol and 67.8% propylene glycol in water.

Microsomal inducer and inhibitor dosage regimens. Treatment regimens for BNF, PCN, and PHE were according to Haugen *et al.* (1976), except that all injections were im instead of ip. For BNF (Aldrich Chemical Co., Inc.), animals were injected with 80 mg/kg in stripped corn oil (Eastman Kodak Co., Rochester, N.Y.) approximately 44 hr before air or O_3 exposure. PCN (gift of the Upjohn Co., Kalamazoo, Mich.) which had first been dissolved in 5% Tween 80 in water was brought to volume with water. Animals received three 50 mg/kg doses of PCN 24 hr apart and were exposed to air or O_3 on the fourth day. Phenobarbital (Sigma Chemical Co.) was dissolved in saline. Treatments consisted of 4 injections at 24-hr intervals followed by air or O_3 exposure a day after the last injection. The first treatment was 30 mg/kg, and the other three were 60 mg/kg. Treatment with PEN consisted of three injections, 24 hr apart, of Nembutal which had been diluted in physiological saline. Doses, which were similar to those of Yamamoto *et al.* (1977), were 50, 75, and then 100 mg/kg. Ozone exposure was conducted about 20 hr after the last treatment.

To examine the influence of inhibitors, SKF 525A (2-diethylaminoethyl-2,2-diphenylvalerate), which was a gift of Smith, Kline, and French, Inc. (Philadelphia, Pa.), and piperonyl butoxide (ICN Pharmaceuticals, Plainview, N.Y.) were used. SKF 525A and piperonyl butoxide were dissolved in saline and stripped corn oil, respectively. Doses were 1.25, 2.5, or 5 mg/kg SKF 525A and 5, 15, or 25 mg/kg piperonyl butoxide. Three doses of an inhibitor used in each daily experiment were injected im immediately before O_3 exposure.

Ozone exposure system. Ozone exposures were conducted as described earlier (Graham *et al.*, 1981). Briefly, mice received whole body exposures in a Rochester-type chamber. On any given day, one group of animals received O_3, while the other received filtered clean air. All O_3 exposures were for 5 hr to 1960 $\mu g\ O_3/m^3$ (1 ppm). Ozone was generated from 100% O_2 (extra dry) with an Orec Model 03V5-0 ozonizer (Ozone Research and Equipment Corp., Phoenix, Ariz.) and monitored continuously with a chemiluminescence O_3 analyzer (Bendix Corp., Ronceveite, W.V.). The O_3 monitor was calibrated twice a month by the gas-phase titration technique (U.S.E.P.A., 1976).

Experimental design and statistical methodology. Techniques used in the analysis of the experiments involved primarily analysis of variance and multiple-comparison procedures. Factors that were analyzed were strain of mice, microsomal monooxygenase inducers, competitive inhibitors, route of injection, vehicle, and exposure to air or O_3. Duncan's (1955) multiple-range test was used to compare pairs for differences in response among doses of the competitive inhibitors for both air- and O_3-exposed mice; Dunnett's test (Dunnett, 1964) was used to compare each treatment to the control. These multiple comparison procedures were used only if the analysis of variance univariate F test was significant in order to control more adequately the experimental error rate (Bernhardson, 1975). For the research reported, a probability level equal to or greater than 0.1 is designated as not statistically significant. For lower probability levels, the actual value or range is given.

RESULTS

Effect of Ozone on Pentobarbital-, Hexobarbital, and Thiopental-Induced Sleeping Times and Zoxazolamine-Induced Paralysis Times

Initially, six groups of CD-1 mice were studied each day: PEN, hexobarbital, and zoxazolamine, with and without O_3. It was observed that hexobarbital treatment resulted in excessive variability of S.T., preventing adequate power for statistical analysis with the sample size used. Therefore, separate experiments were conducted with hexobarbital. For logistical reasons, thiopental was examined separately with either air or O_3.

Ozone significantly increased PEN-induced, hexobarbital-induced, and thiopental-induced S.T. and zoxazolamine-induced paralysis time (Fig. 1). Since it was not intended to study the influence of drug dose on the magnitude of the O_3 effect, it cannot be determined if the O_3 effect was actually greater in the zoxazolamine- or thiopental-injected animals. There was no significant effect of O_3 on I.T. induced by PEN or zoxazolamine. However, with hexobarbital and thiopental, O_3 exposure increased ($p = 0.002$) I.T. by 1.4 and 0.3 min, respectively.

Influence of Mixed-Function Oxidase Inducers on Pentobarbital-Induced Sleeping Time

Before beginning the O_3 exposure experiments, the effects of the vehicles needed to dissolve the MFO inducers were investigated by the same treatment regimens with respect to both type and time of treatment relative to gas exposure and PEN-induced S.T. determinations. All mice were exposed to air for 5 hr before PEN-induced S.T. was determined. When compared to saline injection or no injection, the vehicles caused no significant differences on PEN-induced S.T. For PEN-induced I.T., the only significant difference was that the vehicle (corn oil) for BNF caused a 0.7-min increase ($p < 0.05$) over saline injection in CD-1 mice.

For the inducer studies, four groups of mice were examined on a given day (air-vehicle, air-inducer, O_3-vehicle, and O_3-inducer). In CD-1 mice pretreated with PEN, there was no significant effect of PEN on PEN-induced S.T. (Fig. 2) or I.T. Ozone still caused an increased PEN-induced S.T. Because of the lack of any apparent effect of PEN pretreatment, another higher-dose

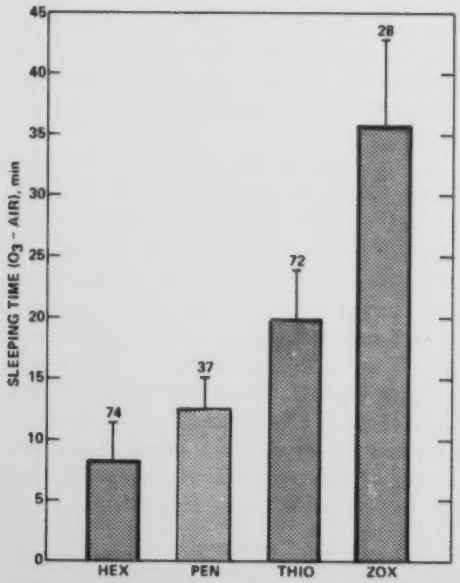

FIG. 1. Effect of 1 ppm $O_3 \times 5$ hr on sleeping or paralysis time induced by hexobarbital (HEX), pentobarbital (PEN), thiopental (THIO), or zoxazolamine (ZOX) in CD-1 female mice. Sample sizes are given above the mean difference bars. The mean difference is greater than zero ($p < 0.05$) for all drugs.

pretreatment regimen was employed. Mice received two injections per day of 50 mg/kg PEN the first day and 75 mg/kg PEN the second and third day before PEN-induced S.T. was measured on the fourth day. Those mice pretreated with PEN ($n = 30$) had a PEN-induced S.T. of 23.0 min ± 1.5 SE, while vehicle-treated animals ($n = 28$) had a PEN-induced S.T. of 24.2 min ± 2.3 SE. The PEN-induced I.T.'s were 4.3 ± 0.3 and 1.3 min ± 0.3 SE for the PEN and vehicle groups, respectively. Thus, even with this increased dosage, there were no statistically significant alterations.

Pretreatment with PHE or PCN caused a reduction in PEN-induced S.T. in the air- and O_3-exposed CD-1 mice (Fig. 2). In both the vehicle- and the inducer-treated animals, O_3 increased PEN-induced S.T. There was no significant difference in the magnitude of the O_3 effect between the vehicle and PHE or PCN treatment groups. Neither O_3 exposure nor PHE or PCN pretreatment altered significantly the PEN-induced I.T.

To determine whether the Ah locus is involved in the response to O_3, additional strains of mice were tested. BNF is a microsomal inducer in C57BL (Ah^b/Ah^b), but not in DBA (Ah^d/Ah^d), mice (Nebert, 1978a,b). Therefore, these strains as well as

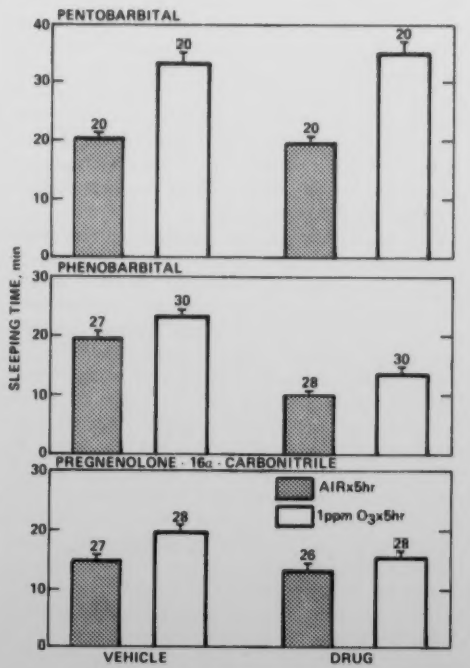

FIG. 2. Influence of MFO inducer pretreatment on the effect of 1 ppm $O_3 \times 5$ hr on pentobarbital-induced sleeping time in female CD-1 mice. Numbers above the standard error bars are sample sizes. See Methods for description of vehicles and dosage regimens. Ozone increased pentobarbital-induced sleeping time, irrespective of drug pretreatment ($p = 0.0001$ for pentobarbital; $p = 0.0006$ for phenobarbital; and $p = 0.0009$ for pregnenolone-16α-carbonitrile). Pentobarbital pretreatment caused no statistically significant ($p > 0.1$) effects, irrespective of type of gas exposure. Phenobarbital and pregnenolone-16α-carbonitrile pretreatment caused a decreased pentobarbital-induced sleeping time ($p = 0.0001$ and 0.005, respectively), irrespective of the type of gas exposure.

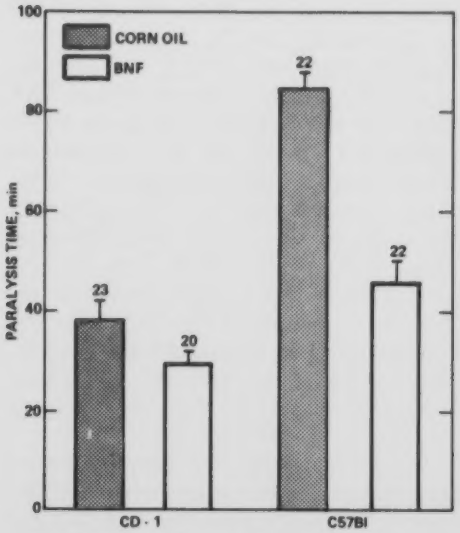

FIG. 3. Effect of β-naphthoflavone (BNF) pretreatment on zoxazolamine-induced paralysis time in female CD-1 and C57B1 mice. See Methods for dosage regimens. Numbers above standard error bars are sample sizes. BNF pretreatment caused a decreased zoxazolamine-induced paralysis time ($p = 0.0001$) in both strains of mice.

CD-1 mice were examined for the O_3 effect. Since the Ah genome of CD-1 mice is not known, a preliminary experiment was conducted to estimate the Ah phenotype by the method recommended by Robinson and Nebert (1974) and Nebert and Felton (1978). For each of the three replicate experiments, four groups of animals, all of which were exposed to air for 5 hr, were used: C57BL, vehicle injected; C57BL, BNF injected; CD-1, vehicle injected; and CD-1, BNF injected. BNF caused a 44 and 34% decrease in zoxazolamine-induced paralysis time in C57BL and CD-1 mice, respectively (Fig. 3). BNF also reduced ($p < 0.05$) zoxazolamine-induced I.T. in CD-1, but not in C57BL mice, from 3.0 ± 0.1 to 2.6 min ± 0.1 SE.

In the experiments to examine the effects of O_3 on three strains of BNF-treated mice, the PEN-induced S.T. results for CD-1 and DBA mice were similar in that O_3 increased, while BNF decreased PEN-induced S.T. (Fig. 4). There were no significant effects on PEN-induced I.T. in CD-1 mice, although a slight increase ($p = 0.08$) in I.T. occurred in BNF treated-DBA mice compared to vehicle-injected mice. Analysis of variance of the data (Fig. 4) for C57BL mice indicated a significant ($p = 0.01$) interaction between drug treatment and gas exposure. Dunnett's test indicated that O_3 increased PEN-induced S.T. in the vehicle group, but

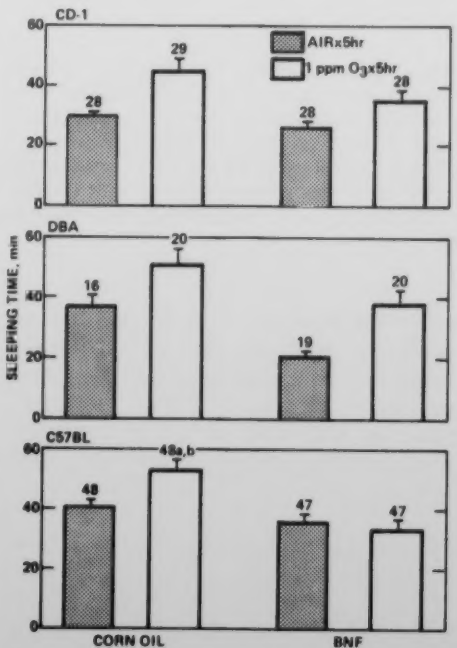

FIG. 4. Influence of β-naphthoflavone (BNF) pretreatment on the effect of 1 ppm $O_3 \times 5$ hr on pentobarbital-induced sleeping time in three strains of mice. See Methods for dosage regimens. Numbers above standard error bars are sample sizes. Ozone increased pentobarbital-induced sleeping time irrespective of corn oil or BNF pretreatment in CD-1 ($p = 0.001$) and DBA ($p = 0.0003$) mice. BNF decreased pentobarbital-induced sleeping time irrespective of type of gas exposure in CD-1 ($p = 0.006$) and DBA ($p = 0.0007$) mice. a = For a given drug treatment, the O_3 mean is different from the air mean ($p < 0.05$). b = For a given type of gas, the corn oil mean is different from the BNF mean ($p < 0.05$).

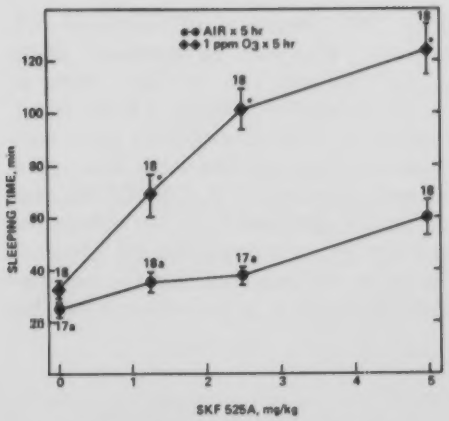

FIG. 5. Influence of SKF 525A on the effect of 1 ppm $O_3 \times 5$ hr on pentobarbital-induced sleeping time in female CD-1 mice. Numbers above standard error bars are sample sizes. *O_3 means are different from the corresponding air means ($p < 0.05$) using Dunnett's test. a = All points sharing this letter are not different from one another ($p > 0.05$) using Duncan's multiple-range test.

not in the BNF group. BNF caused a reduction in PEN-induced S.T., but this decrease was only significant for O_3-exposed animals, which had a 37% lower value. Pretreatment with BNF caused no significant effect on PEN-induced I.T. in C57BL mice.

Effect of Competitive Inhibitors of Mixed-Function Oxidases

To determine whether MFO inhibitors such as SKF 525A or piperonyl butoxide would affect the magnitude of the O_3 effect on PEN-induced S.T., CD-1 mice pretreated with various doses of inhibitors were exposed to either air or O_3. The PEN-induced S.T. data for the SKF 525A study are illustrated in Fig. 5. Analysis of variance indicated a gas (O_3 and/or air) $\times$ SKF 525A dose interaction ($p = 0.0001$) so further analysis was done. In the air-exposed mice, only the 5 mg/kg SKF 525A treatment, which caused a 27% increase over the zero dose, significantly increased the PEN-induced S.T. In the O_3-exposed animals, there was an increase in PEN-induced S.T. for each increasing dose. Only in the presence of SKF 525A was there an effect of O_3 on PEN-induced S.T. The 5 mg/kg treatment resulted in an increased magnitude ($p < 0.05$) of the O_3 effect over the 1.25 mg/kg and zero dose, but not over the 2.5 mg/kg dose. Thus, as the dose of SKF 525A was increased, there was a trend toward an increasing magnitude of the O_3 effect. For PEN-induced I.T., only the 2.5 mg/kg treatment with SKF 525A resulted in a difference (1.3 min decrease in the O_3 group, $p < 0.05$) between the O_3 and air groups.

Animals pretreated with piperonyl butoxide exhibited a trend toward increased PEN-induced S.T. as the dose increased (Fig. 6). O_3 increased PEN-induced S.T. for all doses of piperonyl butoxide; however, this increase was only significant at the 0 (vehicle), 5, and 25 mg/kg treatment. At the highest dose, the effect of O_3 was significantly enhanced ($p < 0.05$) over that at other doses. No sig-

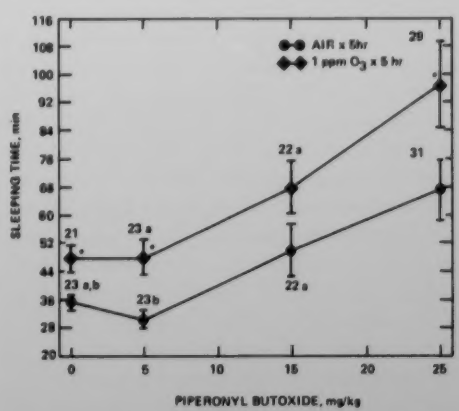

FIG. 6. Influence of piperonyl butoxide on the effect of 1 ppm $O_3 \times 5$ hr on pentobarbital-induced sleeping time in female CD-1 mice. Numbers above standard error bars are sample sizes. *O_3 means are different from the corresponding air means ($p < 0.05$). a or b = For a given gas, all points sharing this letter are not different from one another ($p > 0.05$) using Duncan's multiple-range test.

nificant differences were observed on PEN-induced I.T.

DISCUSSION

Ozone altered the mechanisms of the termination of action of PEN, thiopental, zoxazolamine, and hexobarbital. Within the liver, it appears that PEN and hexobarbital metabolism are more related to cytochrome *P*-450- than to cytochrome *P*-448-dependent activities (Atlas *et al.*, 1976; Jacobson *et al.*, 1972; Robinson and Nebert, 1974; Conney *et al.*, 1960a; Alvares and Kappas, 1977; Noordhoek, 1968). However, some differences exist between hexobarbital- and PEN-induced S.T. For example, aromatic hydrocarbons had no effect on hexobarbital-induced S.T. (Robinson and Nebert, 1974), but did cause a decrease in PEN-induced S.T. in the studies reported here. Zoxazolamine metabolism is more related to cytochrome *P*-448 than to cytochrome *P*-450. From the results with these four drugs, it would appear that O_3 affects some aspects of both drug redistribution and metabolism. Because both aliphatic and aromatic hydroxylation mechanisms and cytochrome *P*-450 and *P*-448 are involved in the termination of action of these drugs, it is possible that several components of the cytochrome *P*-450 system were altered, increasing the likelihood that the effect of O_3 on xenobiotic metabolism is broader than that demonstrated by just these drugs.

While there are some differences in the mechanisms of action of SKF 525A and piperonyl butoxide (Anders, 1968), they are both competitive inhibitors of the cytochrome *P*-450 system, and in the experiments reported herein, they caused an increased PEN-induced S.T. in air-exposed mice. This finding is consistent with the research of others (Blackham and Spencer, 1969; Jori *et al.*, 1970; Conney *et al.*, 1972). Although linear dose–response relationships were not observed, there was a trend toward an increased magnitude of the O_3 effect as the dose of inhibitor was increased. Such a finding might imply an effect of O_3 on the cytochrome *P*-450 system, perhaps the occurrence of non-linear PEN kinetics under conditions of partial enzyme inhibition.

Because of the genetic influence on BNF induction, C57BL and DBA mice were used as well as CD-1 mice in the PEN-induced S.T. studies. Using the technique described by Robinson and Nebert (1974) and Nebert and Felton (1978) for estimation of Ah^b, it was found that both C57BL and CD-1 mice had lowered zoxazolamine-induced paralysis time after BNF treatment. Thus, it can be assumed that the Cd-1 mouse has a phenotype with Ah^b. Given this information, it is difficult to postulate why both CD-1 and DBA mice responded similarly to BNF, with or without O_3. However, the Ah locus relates primarily to aryl hydrocarbon hydroxylase activity and associated events and does not mean that BNF is without effect in the DBA nonresponsive mouse. Also, PEN hydroxylase activity is not associated with the Ah locus (Atlas *et al.*, 1976). The lack of a difference in PEN-induced S.T. between the air- and O_3-exposed BNF-treated C57BL mice is unique in these studies, since O_3 almost always caused an elevated PEN-induced S.T. That the model system was still an effective indicator of O_3 effects is shown by the vehicle-treated mice which had an increased PEN-induced S.T. after O_3 exposure. One possible explanation is that, although C57BL and CD-1 mice appear to share the same Ah phenotype, the genotype may be different. In addition, it can be expected that there are many differences, besides the Ah genotype, among the three strains of mice and one of these unknown factors may account for the observed results.

In the studies reported here for CD-1 mice, PCN, PHE, and BNF pretreatment decreased PEN-induced S.T. compared to the vehicle group in both air- and O_3-exposed mice. Ozone increased S.T. in both the drug and vehicle treatment groups. Also the mag-

nitude of the O_3 effect was not significantly altered by PCN, PHE, or BNF treatment. Preliminary studies with PHE induction (data not reported) suggested that different doses of PHE did not alter the magnitude of the O_3 effect. Even without dose–response data for MFO inducers, it may be assumed that it is unlikely that PHE, BNF, and PCN induced PEN metabolic systems to an equivalent extent. If this assumption were correct, then differing levels and types of induction did not alter the magnitude of the O_3 effect. Therefore, it is suggested that O_3 is decreasing the activity of a common pathway of MFO metabolism which is not affected by inducers in a major way. This reasoning is consistent with the inhibitor studies, in which case, both an inhibitor and O_3 affected fractions of the cytochrome *P*-450 system.

These studies, therefore, suggest that O_3 exposure results in a slowing of the termination of action of drugs which are metabolized by MFO. The effect of O_3 does not appear to be restricted to one specific isozyme of the hepatic cytochrome *P*-450 system as inferred from the inducer studies. Effects on the redistribution of drugs to other compartments of the body aside from the liver apparently occur as indicated by the studies with thiopental. Since O_3 is inhaled, direct effects on the uptake, metabolism, and redistribution of drugs by the lung may be responsible. Alternatively, O_3 exposure may result in the release of pharmacologically active compounds into the circulation, affecting both drug metabolism and uptake.

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